Activity of Four *Salvia* Species Against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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Source: Southwestern Entomologist, 38(1):67-73. 2013.
Published By: Society of Southwestern Entomologists
DOI: http://dx.doi.org/10.3958/059.038.0107
URL: http://www.bioone.org/doi/full/10.3958/059.038.0107

Activity of Four *Salvia* Species against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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Resumen. Se evaluó la actividad insectistática e insecticida de los extractos clorofórmicos de las partes aéreas de cuatro especies de plantas medicinales del Género *Salvia* (Lamiaceae) en el ciclo de vida del gusano cogollero del maíz *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Todos los extractos mostraron ambos tipos de actividad. Los extractos de *Salvia microphylla* Kunth y *Salvia connivens* Epling presentaron una elevada actividad insecticida (LV_{50} 916 y 936 ppm, respectivamente), mientras que los extractos de *Salvia keerlii* Benth., y *Salvia ballotiflora* Benth., mostraron moderada actividad insecticida (LV_{50} 1,527 y 1,685 ppm, respectivamente). *S. connivens* tuvo la mejor actividad insectistática (a 1,000 ppm). Este extracto prolongó las fases larval y pupal en 7.6 y 3.4 d respectivamente, y se observó una disminución en el peso pupal de 30.4%. El extracto de *S. microphylla* incrementó las fases larval y pupal 6.5 y 2.9 d respectivamente y redujo el peso pupal 25.3%. El extracto de *S. keerlii* aumentó la fase larval y pupal 4.9 y 3.1 d respectivamente y disminuyó el peso de las pupas 16.4% y el extracto de *S. ballotiflora* prolongó las fases larval y pupal 5.2 y 2.9 d respectivamente y redujo el peso pupal 13.2%. Estos resultados indican que debido a la actividad insecticida e insectistática contra *S. frugiperda*, estos extractos podrían emplearse para el control de este insecto plaga.

Abstract. Chloroform extracts from the aerial parts of four species of medicinal plants of the genus *Salvia* (Lamiaceae) were tested for insectistatic and insecticidal activities against the life cycle of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). All extracts showed both activities. Extracts from *Salvia microphylla* Kunth and *Salvia connivens* Epling had high insecticidal activity (LV_{50} 916 and 936 ppm, respectively), and *Salvia keerlii* Benth., and *Salvia ballotiflora* Benth., had moderate insecticidal activity (LV_{50} 1,527 and 1,685 ppm, respectively). *S. connivens* had the best insectistatic activity (at 1,000 ppm). This extract prolonged the larval and pupal phases by 7.6 and 3.4 days, respectively, and decreased the pupal weight 30.4%. *S. microphylla* extract increased the larval and pupal phases by 6.5 and 2.9 days, respectively, and reduced the pupal weight

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25.3%. *S. keerlii* extract increased the larval and pupal phases by 4.9 and 3.1 days, respectively, and decreased the pupal weight 16.4%, and the *S. ballotiflora* extract increased the larval and pupal phases by 5.2 and 2.9 days, respectively, and reduced the pupal weight 13.2%. These results indicated that, because of their insecticidal and insectistatic activities, these extracts may be used to control fall armyworm.

Introduction

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is one of the most destructive insect pests of maize, *Zea mays* L., in the tropical and subtropical regions of the Americas (Andrews 1980, 1988). Integrated pest management (IPM) seems to be a good alternative to control noctuid insects. IPM programs combine different control methods that include use of botanical insecticides (Rossetti et al. 2008). Interest in botanicals with activity against insect pests resulted from the need to provide alternatives to reduce the use of synthetic insecticides that can adversely affect the environment (Pavela and Chermenskaya 2004). Most botanical commercial products originated from tropical and subtropical plants. Because of the intensity of plant-insect interactions in tropical and subtropical areas, plants in these regions developed defense mechanisms against insect pests, resulting in promising sources of new insecticidal substances (Prakash and Rao 2000).

The genus *Salvia* (Lamiaceae) includes more than 900 species that grow in temperate and tropical zones around the world (Penso 1983). Plants from this genus have been used for their beneficial healing properties for many years; its name comes from the Latin word for health (S*alvare*) (Rivera et al. 1994).

Some species of this genus have been used as a repellent against Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Phycitinae) (Karahroodi et al. 2009). In addition to repelling the insect, these plants can also induce abnormalities in treated larvae and in pupae and adults (Shoukry et al. 2003). This genus also has insecticidal and genotoxic activities against fruit fly, *Drosophila melanogaster* Meigen and olive fruit fly, *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) larvae (Pavlidou et al. 2004) and insecticidal activity against larvae of cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (Pavela and Chermenskaya 2004).

The aim of the present work was to determine the insectistatic and insecticidal activity of chloroform extracts of the aerial parts of *Salvia ballotiflora* Benth.*, Salvia connivens* Epling*, Salvia keerlii* Benth., and *Salvia microphylla* Kunth against fall armyworm.

Materials and Methods

The aerial parts of *S. ballotiflora* (Voucher SLPM 44561)*, S. connivens* (Voucher SLPM 43013), and *S. keerlii* Benth. (Voucher SLPM 44562) were collected in the Municipio of Guadalcázar, San Luis Potosí, México. Taxonomic authentication was by José García-Pérez at the Isidro Palacios Herbarium of the Universidad Autónoma de San Luis Potosí. *S. microphylla* (Voucher IMSS 15821) was collected in the Municipio of Tenancingo, Estado de México, México. Taxonomic authentication was by M. in Sc. Abigail Contreras of the Centro Médico Siglo XXI Herbario of Instituto Mexicano del Seguro Social.

Dried and powdered aerial parts (300 g leaves and stems) of each *Salvia* species were extracted with 1 liter of chloroform under reflux for 4 hours. The extract was filtered, and the solvent was removed under reduced pressure using a rotatory evaporator. The yields were: *S. ballotiflora* (9.88%), *S. connivens* (5.03%), *S. keerlii* (13.41%), and *S. microphylla* (1.18%).

Each extract was tested for the following phytochemicals: 1) alkaloids: silicotungstic acid, Meyer reagent; 2) coumarins: fluorescence with H_2SO_4; 3) flavonoids: H_2SO_4 concentrate, Dimroth reagent; 4) lignanes: $FeCl_3$, H_2SO_4; 5) limonoids: NaOH, H_2SO_4; 6) quinones: NaOH/HCl, α naftol/MetOH/HCl; 7) sterols: Salkoscky reagent, Liebermann/Burchard reagent; and 8) terpenes: Noller reagent.

Larvae of fall armyworm were reared in the Laboratorio de Investigaión en Química Orgánica of the Departamento de Sistemas Biológicos of the Universidad Autónoma Metropolitana Unidad Xochimilco, at 25 ± 2ºC, 70% relative humidity, and photoperiod of 14:10 light:dark hours according to the methodology and diet of Bergvinson and Kumar (1997).

First-instar fall armyworm larvae were used. Twenty-four larvae were selected randomly for each concentration of plant extract. Preliminary evaluation of each plant extract was at five concentrations between 0.5 and 5,000 ppm. The extract was mixed with the larval diet. Based on results of the preliminary evaluation, the concentration-dependent (500 to 5,000 ppm) effect of the extract was measured according to the methodology of Ramos-López et al. (2010). The effect of the extract was monitored during all larval stages, called the larval-phase duration, and the pupal stage, called the pupal-phase duration. The number of pupae formed (larval viability), number of adults formed (pupal viability), and weight of pupae at 24 hours were assessed. The larval viability (LV_{50}) corresponded to 50% of the larvae of fall armyworm during all larval phases for each extract.

A completely randomized experimental design was used. ANOVA and the Tukey test were used, and the LV_{50} was calculated using the SYSTAT statistical analysis program (Ato et al. 1990).

Results

The *S. ballotiflora* extract tested positive for cumarins, flavonoids, and lignanes (Table 1). The *S. connivens* extract tested positive for cumarins, flavonoids, and sterols. The*S. keerlii* extract tested positive for flavonoids, lignanes, and sterols, and the *S. microphylla* extract tested positive for alkaloids, cumarins, flavonoids, and limonoids.

In the insecticidal activity assays, exposure to the chloroform extract of the aerial parts of *S. ballotiflora* (Table 2) induced 12.5, 25.0, 41.7, and 62.5% of larval viability at 5,000, 4,000, 2,000, and 1,000 ppm, respectively. These values indicated the average number of pupae formed. Pupal viability, which represents the average number of adults that emerged, was 8.3, 12.5, 29.2, and 45.8% at 5,000, 4,000, 2,000, and 1,000 ppm. The LV_{50} was 1,685 ppm. The *S. connivens* extract (Table 3) caused larval viability of 8.5, 20.8, 33.3, 37.5, and 62.5% at 5,000, 4,000, 2,000, 1,000, and 500 ppm. Pupal viability was 20.8, 25.0, and 58.3% at 2,000, 1,000, and 500 ppm, respectively. The LV_{50} was 936 ppm. The larval viability with the *S. keerlii* extract (Table 4) was 12.5, 16.7, 37.5, and 58.3% at 5,000, 4,000, 2,000, and 1,000 ppm, respectively, and pupal viability was 4.2, 4.2, 25.0, 37.5, and 62.5% at 5,000, 4,000, 2,000, 1,000, and 500 ppm, respectively. The LV_{50} was 1,527 ppm. The larval viability with the *S. microphylla* extract (Table

Table 1. Phytochemical Test of the Chloroform Extract of *S. ballotiflora, S. connivens, S. keerlii*, and *S. microphylla*

Extract	SILICOT	ALK MEYER	H_2SO_4	COU NaOH/HCl	H_2SO_4	FLAV DIMROTH	$FeCl_3$	H_2SO_4	LIG NaOH	H_2SO_4	LIM NaOH/HCl	QUI α-aftol/MeOH	LIEBERM	STE /BURCH	TERP NOLLER
S. ballotiflora	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-
S. connivens	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-
S. keerlii	-	-	-	-	+	-	+	+	-	-	-	-	-	+	-
S. microphylla	-	+	+	-	+	-	-	-	-	+	-	-	-	-	-

ALK = alkaloids; COU = coumarins; FLAV = flavonoids; LIG = lignanes; LIM = limonoids; QUI = quinones; STE = sterols; TERP = terpenes; SILICOT = silicotungstic acid; TRICHL = trichloroacetic.

Table 2. Larval and Pupal Viability, Larval and Pupal Duration, and Pupal Weight of *S. frugiperda* with Chloroform Extract of *S. ballotiflora*

Concentrate	Viability (%)		Duration (days)		Pupal weight
(ppm)	Larvae	Pupae	Larvae	Pupae	(mg)
5,000	12.5 ± 6.9*	8.3 ± 5.8*	35.0 ± 2.9*	15.0 ± 1.0*	145.0 ± 22.7*
4,000	25.0 ± 9.0*	12.5 ± 6.9*	30.8 ± 0.9*	14.0 ± 0.6*	156.5 ± 16.6*
2,000	41.7 ± 10.3*	29.2 ± 9.5*	29.2 ± 0.7*	13.0 ± 0.2*	174.2 ± 10.2*
1,000	62.5 ± 10.1*	45.8 ± 10.4*	27.0 ± 0.5*	12.5 ± 0.2*	200.6 ± 6.2*
500	75.0 ± 9.0	66.7 ± 9.8	23.5 ± 0.8	10.8 ± 0.3*	221.4 ± 3.6
0	91.7 ± 5.8	87.5 ± 6.9	21.8 ± 0.6	9.6 ± 0.2	231.2 ± 4.2
LV_{50}	1.685×10^3	ppm			

Results are the mean of at least 24 measurements ± standard error; LV_{50} was calculated using larval viability; * = significantly different from the check at $P < 0.05$.

Table 3. Larval and Pupal Viability, Larval and Pupal Duration, and Pupal Weight of *S. frugiperda* with Chloroform Extract of *S. connivens*

Concentrate	Viability (%)		Duration (days)		Pupal weight
(ppm)	Larvae	Pupae	Larvae	Pupae	(mg)
5,000	8.3 ± 5.8*	0*	56.5 ± 4.5*	-	98.0 ± 3.0*
4,000	20.8 ± 8.5*	0*	50.0 ± 1.0*	-	100.3 ± 5.4*
2,000	33.3 ± 9.8*	20.8 ± 8.5*	30.3 ± 1.9*	13.0 ± 0.5*	144.4 ± 8.3*
1,000	37.5 ± 10.1*	25.0 ± 9.0*	29.4 ± 0.8*	11.0 ± 0.4*	161.0 ± 14.8*
500	62.5 ± 10.1*	58.3 ± 10.3*	23.0 ± 1.2	9.9 ± 0.2	208.4 ± 10.4*
0	91.7 ± 5.8	87.5 ± 6.9	21.8 ± 0.6	9.6 ± 0.2	231.2 ± 4.2
LV_{50}	0.936×10^3	Ppm			

Results are the mean of at least 24 measurements ± standard error; LV_{50} was calculated using larval viability; * = significantly different from the check at $P < 0.05$.

Table 4. Larval and Pupal Viability, Larval and Pupal Duration, and Pupal Weight of *S. frugiperda* with Chloroform Extract of *S. keerlii*

Concentrate	Viability (%)		Duration (days)		Pupal weight
(ppm)	Larvae	Pupae	Larvae	Pupae	(mg)
5,000	12.5 ± 6.9*	4.2 ± ND*	32.0 ± 0.6*	14.0 ± ND*	126.0 ± 24.1*
4,000	16.7 ± 7.7*	4.2 ± ND*	31.3 ± 1.3*	13.0 ± ND*	141.8 ± 16.8*
2,000	37.5 ± 10.1*	25.0 ± 9.0*	27.7 ± 0.6*	12.8 ± 0.3*	162.3 ± 18.1*
1,000	58.3 ± 10.3*	37.5 ± 10.1*	26.7 ± 0.6*	12.7 ± 0.2*	193.3 ± 7.2*
500	79.2 ± 8.5	62.5 ± 10.1*	24.4 ± 0.6*	11.9 ± 0.2*	217.2 ± 4.0
0	91.7 ± 5.8	87.5 ± 6.9	21.8 ± 0.6	9.6 ± 0.2	231.2 ± 4.2
LV_{50}	1.527×10^3	ppm			

Results are the mean of at least 24 measurements ± standard error; LV_{50} was calculated using larval viability; * = significantly different from the check at $P < 0.05$.

5) was 0% at 5,000 ppm; at 4,000, 2,000, 1,000, and 500 ppm, the larval viability rates were 4.2, 29.2, 41.7, and 62.5%, respectively. The LV_{50} was 916 ppm.

The insectistatic activity of the *S. ballotiflora* extract (Table 2) lengthened the larval phase by 13.2, 9.0, 7.4, and 5.2 days at 5,000, 4,000, 2,000, and 1,000 ppm, respectively, lengthened the pupal phase by 5.4, 4.4, 3.4, 2.9, and 1.2 days at 5,000, 4,000, 2,000, 1,000, and 500 ppm, respectively, and reduced the pupal weight

Table 5. Larval and Pupal Viability, Larval and Pupal Duration, and Pupal Weight of *S. frugiperda* with Chloroform Extract of *S. microphylla*

Concentrate	Viability (%)		Duration (days)		Pupal weight
(ppm)	Larvae	Pupae	Larvae	Pupae	(mg)
5,000	0*	-	-	-	-
4,000	4.2 ± ND*	0*	34.0 ± ND*	-	114.0 ± ND*
2,000	29.2 ± 9.5*	12.5 ± 6.9*	30.7 ± 1.0*	14.0 ± 0.6*	157.3 ± 7.9*
1,000	41.7 ± 10.3*	16.7 ± 7.8*	28.3 ± 1.0*	12.5 ± 0.3*	172.7 ± 12.3*
500	62.5 ± 10.1*	50.0 ± 10.4*	23.8 ± 0.7	11.6 ± 0.3*	202.7 ± 9.3*
0	91.7 ± 5.8	87.5 ± 6.9	21.8 ± 0.6	9.6 ± 0.2	231.2 ± 4.2
LV_{50}	0.916×10^3 ppm				

Results are the mean of at least 24 measurements ± standard error; LV_{50} was calculated using larval viability; * = significantly different from the check at $P < 0.05$.

by 37.3, 32.3, 24.7, and 13.2% compared with the check weight at 5,000, 4,000, 2,000, and 1,000 ppm, respectively. The *S. connivens* extract (Table 3) prolonged the larval phase by 34.7, 28.2, 8.5, and 7.6 days at 5,000, 4,000, 2,000, and 1,000 ppm, respectively, prolonged the pupal phase by 3.4 and 1.4 days at 1,000 and 500 ppm, respectively, and reduced the pupal weight by 57.6, 56.6, 37.5, 30.4, and 9.9% compared with the check weight at 5,000, 4,000, 2,000, 1,000, and 500 ppm, respectively. The *S. keerlii* extract (Table 4) extended the larval phase 10.8, 9.5, 5.9, 4.9, and 2.6 days and extended the pupal phase 4.4, 3.4, 3.2, 3.1, and 2.3 days at 5,000, 4,000, 2,000, 1,000, and 500 ppm, respectively. This extract reduced the pupal weight by 40.9, 38.7, 29.8 and 16.4% at 5,000, 4,000, 2,000, and 1,000 ppm. The *S. microphylla* extract (Table 5) increased the larval phase by 12.2, 8.9, and 6.5 days at 4,000, 2,000, and 1,000 ppm, respectively, increased the pupal phase by 4.4, 2.9, and 2.0 days at 2,000, 1,000, and 500 ppm, and decreased the pupal weight 50.7, 32.0, 25.3, and 12.3% at 4,000, 2,000, 1,000, and 500 ppm, respectively.

Discussion

S. microphylla and *S. connivens* extracts had strong insecticidal activity, whereas *S. keerlii* and *S. ballotiflora* extracts had moderate insecticidal activity against fall armyworm. Rashid et al. (2009) reported that dichloromethane extract of the aerial parts of *Salvia cabulica* Benth., resulted in 80% mortality of adult red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), when 10 insects were placed into dishes with a sample of 200 mg of extract dissolved in 3 ml of acetone that was prepared 24 hours before the experiment. The essential oils of *Salvia limbata* C.A. Meyer and *Salvia nemorosa* L. resulted in average mortality rates for granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) (10 and 14%, respectively) at 76.91 µl l^{-1} air concentration. The mortality rate increased with the concentration of essential oils and exposure period (Yildrim et al. 2005). The essential oils from *Salvia hydrangea* DC ex Benth.*, S. nemerosa, Salvia multicaulis* Vahl, and *Salvia sclarea* L. were also tested against granary weevil adults. These essential oils induced 67.33, 39.73, 55.21, and 41.76% mortality at 153.84 µl l^{-1} air concentration (Yildirim et al. 2011). Ramírez-Moreno et al. (2001) reported that 5% aqueous extract of aerial parts of *Salvia karwinskii* Benth., and *Salvia polystachya* Ortega had low insecticidal activity against common green-eyed

white, *Leptophobia aripa elodia* (Boisduval) (Lepidoptera: Pieridae) larvae; they observed a mortality rate of 13% with both plant species.

The four *Salvia* extracts were insectistatic against fall armyworm larvae. Each extract at a concentration of 1,000 ppm increased the larval and pupal phases and decreased the pupal weight. Karahroodi et al. (2009) used an olfactometer and showed 56 and 32% repellency with essential oils of *Salvia multicaulis* Vahl and *Salvia officinalis* L., respectively, against female adults of Indianmeal moth at 15.39 µl l^{-1} air concentration. Additionally, Ramírez-Moreno et al. (2001) observed 7% repellency in 5% aqueous extracts of *S. karwinskii* and *S. polystachya* against *L. aripa elodia* larvae. Lakshmanan et al. (2012) reported antifeedant activity rates of 85.56% by armyworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), 45.64% by corn earworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and 79.45% by croton caterpillar, *Achaea janata* (L.) (Lepidoptera: Noctuidae), with 1,000 ppm of *S. officinalis* essential oil.

This work showed the potential use of extracts from four *Salvia* species against fall armyworm larvae. *Salvia* contains bioactive compounds such as flavonoids, essential oils, diterpenes, and triterpenes, which can act as antifeedants (Tomás-Barberan and Wollenweber 1990). The use of synthetic insecticides can have unwanted consequences such as residues, resistance, and damage to the environment and human health (Yu et al. 2003). Biologically active compounds of plants are assumed to be environmentally more acceptable and less hazardous to humans (Yildirim et al. 2011). To our knowledge, this is the first report to show insecticidal and insectistatic activities of the chloroform extract of aerial parts of *S. ballotiflora, S. connivens, S. keerlii*, and *S. microphylla* against fall armyworm larvae.

Acknowledgment

The authors gratefully acknowledge the Universidad Autónoma Metropolitana unidad Xochimilco for the postdoctoral fellows program scholarship; Osvaldo Campos Rivera, Joselby Martínez Gutiérrez, and Carina de Ramón Garrido for their technical support; and José García-Pérez and Abigail Contreras for the taxonomic identification.

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